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Objectives:

Our goal is to test the Snyder-Dolan classification scheme to see if it can successfully predict similarity and differences in stationary phase behavior for **a wide variety of drugs** under isocratic and **gradient conditions** and more importantly to apply the Synder-Dolan scheme to find "extreme" phases to enable **"orthogonal" separations** for screening for illicit drugs.

Background:

A. Why we need to understand column chemical selectivity?

Reversed phase liquid chromatography (RPLC) is the most powerful analytical methodology for drug analysis. To identify unknown drugs we have proposed running each sample and determining their retention time on a judiciously chosen set of *very different stationary phases*. Thus a method for choosing maximally different phases is very important.

B. Snyder-Dolan Hydrophobic Subtraction Model of RPLC phase selectivity

1. Theory

A lot of work has been done to try to understand column chemical selectivity. One of the most successful models is the "Hydrophobic-Subtraction Model" developed by Lloyd Snyder and his associates. Based on the use of 16 very carefully selected but chemically simple probe solutes Snyder et al. have characterized the selectivity of reversed phase columns using a set of 5 parameters by fitting the retention data of these 16 solutes to the equation:

$$\text{Log } k'_i/k'_{ref.} = \text{H}\eta_i - \text{S}'\sigma_i + \text{A}\beta_i + \text{B}\alpha_i + \text{C}\kappa_i$$

These five parameters represent the five most common solute-column interactions, specifically: hydrophobicity (H), steric resistance (S*), hydrogen-bond acidity (A), hydrogen-bond basicity (B), cation-exchange activity (C). Application of this model can give us a better understanding of nature and relative importance of different solute–column interactions. Thus far, over 350 different commercial RPLC materials have been studied by this method and virtually every phase of every major producer has been characterized.

2. Application

The huge advantage of this model over previous methods is that a single parameter called the "column selectivity function F_s" has been defined, which can be used to quantitatively compare the selectivity of two columns;

$$F_s = \{[12.5(H_2 - H_1)]^2 + [100(S_2 - S_1)]^2 + [30(A_2 - A_1)]^2 + [143(B_2 - B_1)]^2 + [83(C_2 - C_1)]^2\}^{1/2}$$

It is based upon the assumption that differences in selectivity for any two columns can be measured by the distance between the two points in this five parameter multi-dimensional space. Therefore, the smaller the distance (i.e. F_s), the more similar two columns are. In the extreme case when two columns are so close ($F \leq 3$), two columns can be considered essentially "equivalent". On the other hand, columns with bigger F_s are more widely separated; correspondingly they are more different in terms of selectivity. This turns out to be the most

important application of this model since it allows an easy selection of phases which are nearly interchangeable and conversely phases that are dramatically different, both of which are very useful in developing new analytical methods.

C. Reversed phase selectivity triangle

In addition to Snyder's Hydrophobic Subtraction Model, a novel type of phase classification "triangle" was also used in our study for the selection of columns. This approach was recently developed in our lab based on Snyder's Hydrophobic Subtraction Model. For present purposes the details of derivation will not be reviewed here. What is important to know is that we found there are only a few drugs separated by RPLC that are strong hydrogen bond acceptors. This means that the A term is relatively unimportant and can be ignored. Hence by further normalizing the S*， B and C terms by H, we were able to obtain a set of only three significant parameters which classify the properties of stationary phases. The results can be represented in a "selectivity triangle", wherein the apices of the triangle represent the relative contributions of steric hindrance (χ_S), hydrogen bonding basicity (χ_B) and cation exchange capacity (χ_C) to selectivity. As clearly shown in Figure 1, the virtue of this method is that it allows the visualization of the column selectivity by allowing three-dimensional data to be presented in a two-dimensional space. Thus it provides us with a much more informative yet universal approach for phase classification compared to the previous models. With this model, selection of columns of either equivalent or different selectivity is readily achievable which should further facilitate the application of RPLC.

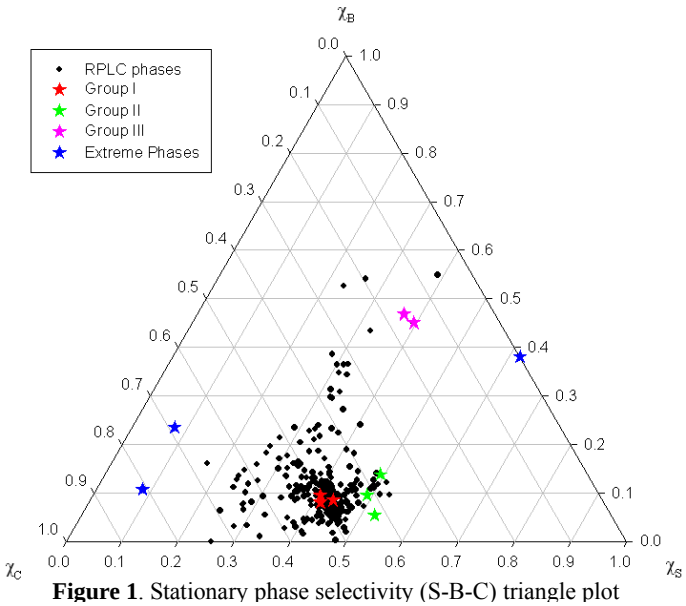

Figure 1. Stationary phase selectivity (S-B-C) triangle plot

Results and Discussion

1. Selection of columns

Based on Snyder's F_s values, we selected 12 columns for our study. As shown in Table I, the first three groups (see sets I, II, and III in Table 1) are each comprised of reversed phases that are within the set similar in terms of Snyder's parameters but different between sets; while the last row shows a group of phases which are very different from one another (i.e. orthogonal). The similarity and difference of these columns were also verified with above triangle scheme to ensure we do pick the right columns.

Table 1. Columns for this study

Set Type	Column A	Column B	Column C	Column D
I (Similar)	Zorbax SB C18 **-Agilent**	ACE 5 C18 **-Mac Mod**	Discovery C18 **-Supelco**	Alltima HP C18 **-Alltech**
II (Similar)	Beta Basic Phenyl **-Thermo**	Prontosil 60-5-Phenyl **-Bischoff**	Zorbax Phenyl **-Agilent**	
III (Similar)	Hypersil Prism C18 **-Thermo**	Bonus RP **-Agilent**		

IV (Different)	ZirChrom PS **-ZirChrom**	Nova Pak CN HP-60[a] **-Waters**	Inertsil CN **-GL Science**	

2. Column selectivity test with drugs under isocratic condition

Before doing the test with the drugs, we ran the 16 Snyder test solutes on all of the above columns. The results proved that the phases we chose were well fit with Snyder's scheme and that the columns were behaving properly. We then wanted to test the Snyder scheme to see if it could successfully predict similarity and differences in phase behavior for a wide variety of drugs. First, 25 drugs were chosen as our probe solutes from over 70 drugs based on their retention times and the UV spectra. Five mixtures were prepared in Snyder's mobile phase (Table 2) and used to test all columns.

Table 2. Drug mixtures

Mixture #1	**Mixture #2**	**Mixture #3**	**Mixture #4**	**Mixture #5**
Zolpidem	Chlorpheniramine	Methapyrilene	Pyrilamine	Chlordiazepoxide
Aminoflunitrazepam	Desipramine	Perphenazine	Bromazepam	Amitriptyline
Oxazepam	Halazepam	Clonazepam	Desalkylflurazepam	Nitrazepam
Estazolam	Temazepam	Triazolam	Nordiazepam	Clobazam
Lormetazepam	Diazepam	Flunitrazepam	Prazepam	Buclizine

All columns were tested under Snyder eluent conditions (50/50 acetonitrile/water with 30 mM phosphate buffer pH 2.8). To compare column selectivity, the standard errors (SE) of retention plot (log k' plot) were obtained for a given column against Discovery C18. The smaller SE is, the more similar two columns are, and vice versa. Figure 2 shows the SE plot for 16 Snyder solutes vs. 18 drugs under isocratic. The very good correlation (R^2=0.919) indicates that the phase behavior for the drugs is really rather well correlated with the results for Snyder's 16 solutes. Therefore, the Snyder-Dolan scheme does reasonably allow us to choose columns with selectivities for drugs. That is the similar columns are still similar; the different columns are indeed radically different in term of the drug solutes.

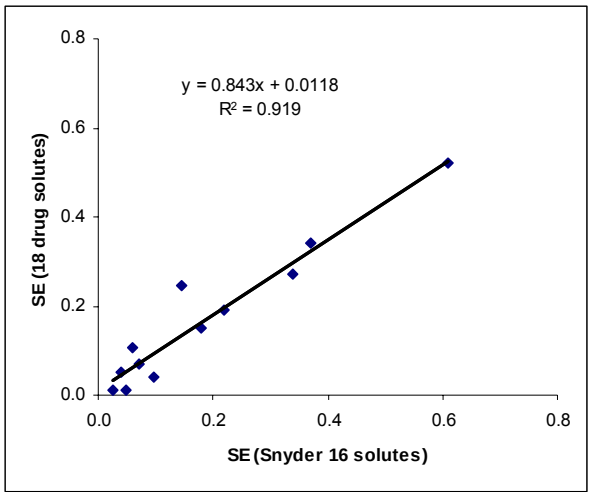

Figure 2. SE plot for Snyder 16 solutes vs. 18 drugs.

3. Column selectivity test with drugs under gradient

Since Snyder's work was all isocratic, it is interesting and very important for our drug screening work to know whether his model works under gradient elution or not. All columns were then tested with 25 drugs under gradient elution. To obtain the proper gradient conditions, we needed to adjust the gradient range and gradient time (t_G) to allow the retention times (t_R) of all drugs fall within the gradient window. The proper gradient conditions used for the drug solutes are: gradient range from 20~70% ACN/buffer (30 mM, pH 2.8); t_G = 10 min; flow rate = 1 mL/min; temperature = 35 ^{0}C.

Under these conditions, the retention times of 25 drugs on all columns were measured. The plots of normalized retention time of one column against another were used to compare column selectivity. Figure 3 shows an example of columns in group IV vs. a Discovery C18 column. We can see that there is a small SE (0.07) for HC-OH vs. Discovery C18 indicating that HC-OH is similar to Discovery C18, which is consistent with a small Snyder's F_s value (23.7) for these two columns. We can also see that the SE values between other three columns (Inertsil CN, Nova Pak CN, and HC-SO_3^-) and Discovery C18 are all very large, which tells us these three columns are very different from Discovery C18, especially Nova Pak CN and HC-SO_3^-, which are extremely different from Discovery C18. These results are consistent with those from our selectivity triangle. *To our knowledge, this is the first time anyone has looked at the Snyder-Dolan classification scheme with the drug solutes under gradient conditions.*

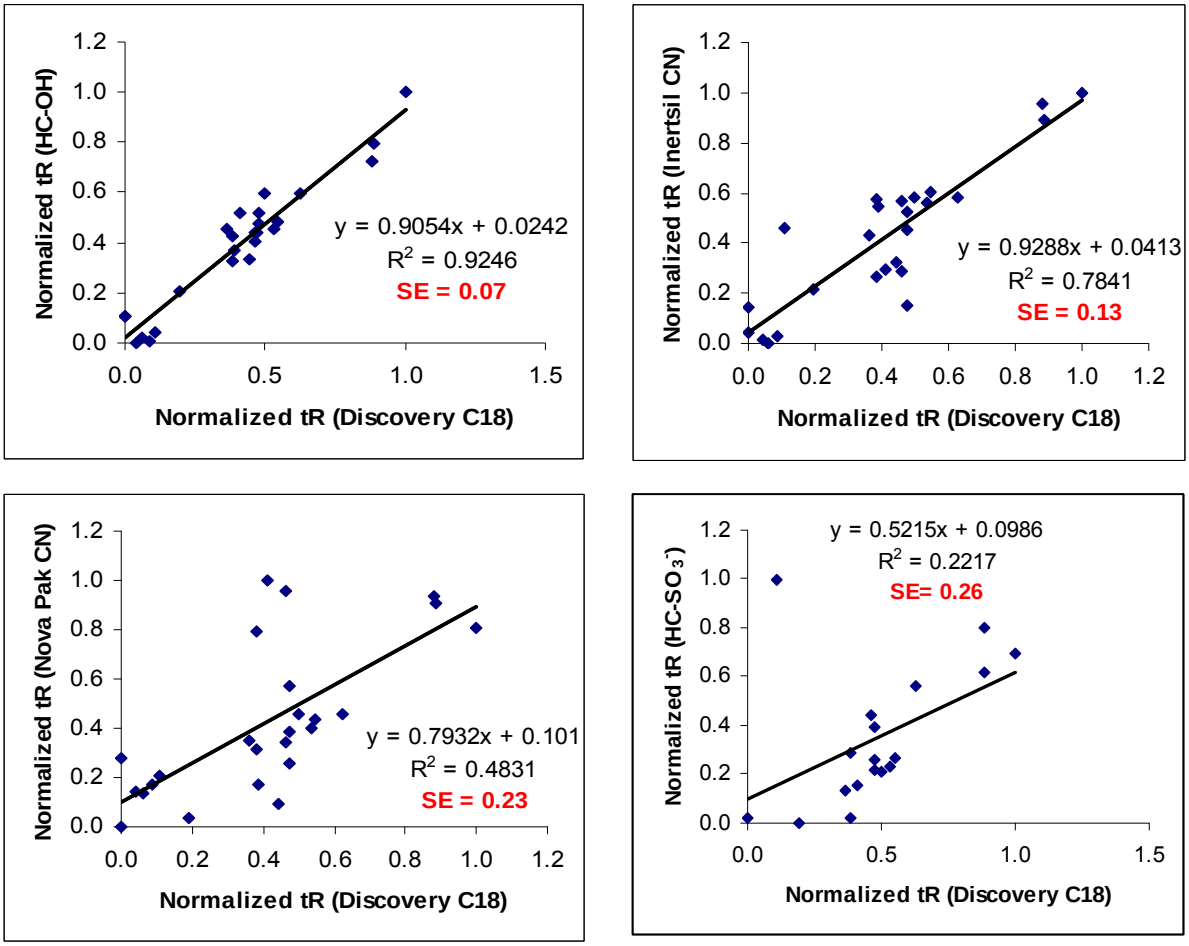

Figure 3. Normalized tR plots for the columns in group IV vs. Discovery C18 under gradient conditions.

(t_G = 10 min; gradient range: 20-70% ACN/buffer; flow rate = 1.0 ml/min; temperature = 35 ^{0}C)

4. Dead volume (V_m) effect on column selectivity under gradient elution

When one transfers one gradient elution method from one instrument to another or same instrument but different column size. The different dwell volume (V_D) of instruments or the different V_m of columns will affect selectivity. To successfully transfer one gradient method to

another instrument or another column, one must adjust conditions to ensure the following two ratios are constant at the same time, which means: when V_D is changed, one must change V_m proportionately to avoid the change of selectivity; when V_m is changed, one must change flow rate (F) or gradient time (t_G) to keep these ratios constant.

$$\text{constant} = \frac{V_D}{V_m} \qquad\qquad \text{constant} = \frac{V_m}{Ft_G}$$

In our work, all 12 columns, from various manufacturers, have slightly different V_m values; therefore, we need to make sure that differences in V_m are not having a big effect on selectivity as measured by SE. Figure 4, 5 shows the effect of V_m adjustment on column selectivity of ACE C18 and Prism RP vs. SB C18, respectively. We can see that, compared to SB C18, there is not any change for the selectivity of ACE C18 or Prism RP with adjusted dwell volumes. Therefore, *it is not necessary to adjust dwell volume for the difference in dead volume for different columns. We can use unadjusted retention time for column comparisons*.

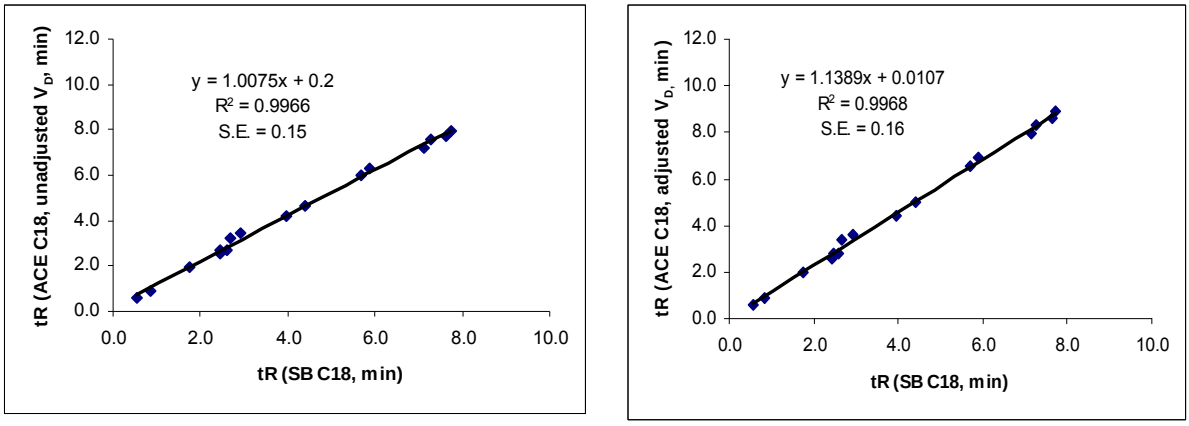

Figure 4. Effect of dead volume on selectivity of ACE C18 under gradient conditions.

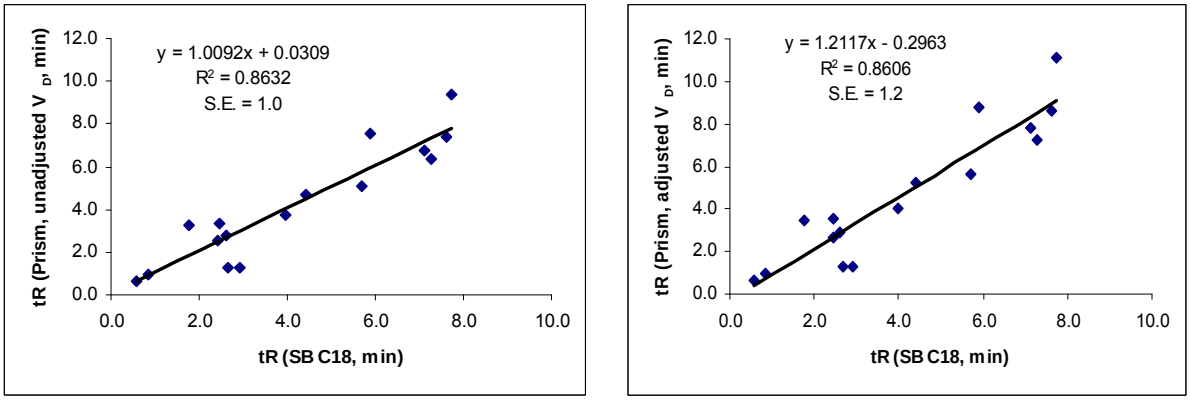

Figure 5. Effect of dead volume on selectivity of Prism RP under gradient conditions.

Conclusions:

In this study, we have tested and compared the selectivity of 12 columns for drugs under both isocratic and gradient elution and came out the following conclusions.

1. We can now confidently use the Snyder-Dolan database of 350 columns to reliably choose similar and very different columns for drugs.
2. The Snyder-Dolan approach can be used with gradient elution data for the drugs.
3. Small changes in dead volume have little effect on column selectivity as measured by the s.e.. Unadjusted retention time can be used directly for column comparisons.
4. The selectivity triangle model based on Snyder's parameters is quite useful for picking maximally orthogonal columns for the drugs.